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Evaluation of diosgenin, a bioactive compound from natural source of *Dioscorea* species: A wild edible tuber plant

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Abstract
Dioscorea is a genus of over 600 species in the family Dioscoreace distributed throughout the tropical and warm temperate regions of the world. The members of the genus *Dioscorea* are one of the oldest tuber crop cultivated or harvested from wild in the tropical region throughout the world and constitute one of the major food item for many ethnic groups. India having rich plant biodiversity possesses about 50 species of *Dioscorea* and among them some have excellent medicinal and nutritional properties. *Dioscorea*, popularly known as Yam worldwide and as Ban Aalu in Odisha, India, is a prime staple medicinal-food substitute for the majority of rural and local people staying in forests of Eastern ghats of Odisha. There are about 13 type of *Dioscorea* species found in Eastern ghats of Odisha. Diosgenin, a commercially important bioactive sapogenin, extracted from tubers belonging to *Dioscorea* species, is used as a precursor in manufacturing of sex hormones, oral contraceptives and other pharmaceutically important steroidal drugs. With the view to commercially exploit a new source of diosgenin, lesser explored tubers of seven species of *Dioscorea* namely; *D. bulbifera* L.(2), *D. pentaphylla* L.(5), *D. hispida* Dennst.(1), *D. alata* L. (3), *D. oppositifolia* L. (2), *D. pubera* Blume (2), *D.hamiltonii* Hook. f.(1) were collected from Eastern Ghats of Odisha through explorations. The diosgenin content was determined in dried tuber by acidic hydrolysis of the glycosides and subsequent extraction with an organic solvent, followed by the HPTLC analysis. HPTLC mobile phase was optimized to toluene: ethyl acetate: formic acid (6: 5:1 v/v), and diosgenin content was found in the range of 0.001 to 0.003% on dry weight basis. The lower diosgenin content presently detected in the species may be due to the interaction of environmental factors or may be due to genetic makeup of the parent clones.

Keywords: Bioactive Compound, *Dioscorea*, Diosgenin, Ethnobotany, HPTLC, Yams, Ethonpharmacology

Introduction
India is a harbor of biodiversity in general and phyto diversity in particular. A number of wild crops remain unexplored in this world and among them some have excellent medicinal and nutritional properties. The plant diversity is distributed from the Western Ghats to Eastern Ghats, along with the North-Eastern region and from the Greater Himalayas to the plains of Ganga. Among these distributed floral regions of the country, the Eastern Ghats are important due to their rich floral diversity [1]. The forests of Odisha form a major part of Eastern Ghats in general and are inhabited by many local communities [2]. They are dependent on the forests for their food and medicine needs. *Dioscorea* is one such tuber crop of family Dioscoreaceae, having maximum use among the local people for food and medicine [3]. However, less documentation and no specific reports are available on the food and medicinal values of these new species. Several wild and cultivated species of *Dioscorea* are known to contain diosgenin. However, all species are not commercially acceptable either for difficulties in cultivation or for low diosgenin content. Diosgenin is a very important and versatile precursor and accounts for about 50 per cent of the total steroid drug output in the world. Diosgenin is being used as a primary product in the synthesis of sex hormones, corticosteroids and in the production of family planning drugs.
Dioscorea, popularly known as Yam worldwide and as Ban Aalu in Odisha, India, is a prime staple medicinal - food substitute for the majority of rural and local people of the state of India. There are about 13 type of *Dioscorea* species found in Eastern ghats of Odisha. The most common *Dioscorea* species are *D. bulbifera* L. (Pita aalu), *D. pentaphylla* L. (Panja Sanga), *D. hispida* Dennst. (Paani aalu), *D. alata* L. (Khamba aalu), *D. oppositifolia* L. (Paani aalu), *D. pubera* Blume (Kukai Sanga) etc. Ten species are known to be bitter in taste or unpalatable when raw. The rural and local people who use them as food supplements make them edible by different traditional practices. *Dioscorea* species tubers are mostly soaked overnight in water

or left overnight in stream and subjected to successive boiling to remove the bitterness [2, 3]. *Dioscorea* species with nutritive and antioxidant content not only enrich the diet of the local rural and local people but also make them ethno medicinally important [4-9].Tubers of different species of *Dioscorea* are used for curing diseases and ailments in different formulations. In India, the most common *Dioscorea* species is *D. alata* L. (Khambha Aalu) and *D. bulbifera* L. (Pita Aalu) distributed in forest patches of Eastern Ghats to the lower Himalayas in the Indian subcontinent throughout the country [2, 3].

Apart from the traditional importance as starchy staple food (such as *D. opposite, D. alata* and *D. japonica*), some *Dioscorea* species are known and used as a source for the steroidal sapogenin, a precursor for the synthesis of steroid drugs[4, 5]. *D. deltoidea* Wall. is the major species exploited in India for diosgenin production from rhizomes. However, the reserves of wild *Dioscorea* plants continue to decline, because of the extensive harvesting and increasingly ecological damage. Yet the failure of achieving fully chemical synthesis of steroids until now has again made *Dioscorea* a very attractive source for steroidal steroidal precursors. China and Mexico are the top two countries with the richest yam resource in the world, the yield of diosgenin accounts for 67% of world production. Therefore, to overcome the shortage of raw materials and to support sustainable development of the pharmaceutical industry associated with diosgenin, a preliminary screening of the different species locally available become necessary to identified new sources of diosgenin for commercial use. Since less documentation is available on the *Dioscorea* species and their traditional uses, the present study was focused on the ethnobotany and pharmacological values of these species along nutraceutical importance.

Ethnobotanical uses: The *Dioscorea* species are important source of secondary staple food as its tubers, commonly known as 'yam' has high nutritional value. Yams have a reputed place in traditional herbal medicinal practices, especially due to their potency in enhancing fertility in males. *Dioscorea* species are rich source of diosgenin, a steroidal sapogenin used as the precursors for corticosteroids and anticonceptional hormones. Several wild and cultivated species of *Dioscorea* are used for treatment of leprosy, dyspepsia, dysentery, psoriasis etc. (Table 1). Many species are used in ayurvedic, unani and other systems of medicine. It is used as purgatives, laxatives, expectorants and for the treatment of poison bites and skin diseases. The present investigation is based on seven species of *Dioscorea* that are found in Odisha. They are enumerated below along with their botanical names; common names; habitat; brief description; biological status; parts used together with ethno botanical and ethno medicinal uses (Table 1)

Active compound- diosgenin: *Dioscorea* species are rich source of sapogenins, especially diosgenin and *D. composite* and *D. floribunda* are widely exploited as source of diosgenin. Diosgenin (Fig. 1) is a bioactive steroidal sapogenin belonging to the triterpene group and is great interest to the pharmaceutical industry. It is the aglycone formed by the hydrolysis of saponin dioscin, a compound found in *Dioscorea spp*. It serves as an important starting material for the production of corticosteroids, sexual hormones, oral contraceptives as well as other steroidal drugs [3-5].

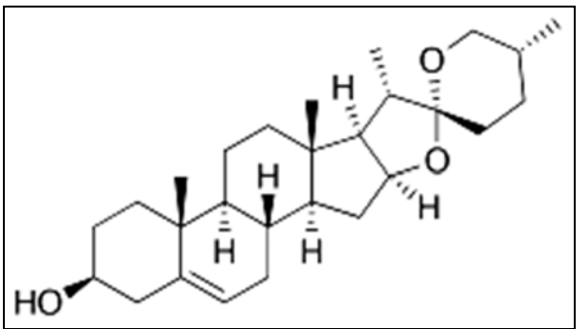

Fig 1: Chemical structure of diosgenin – steroidal sapogenin

It is interesting to note that no appreciable amount of diosgenin has been found in Old World or temperate region *Dioscorea* species. Further, steroidal saponins in *Dioscorea* species have already been used as standard marker compounds in botanical products due to their chemotaxonomical significance and their important biological activities. Different chemical and biological protocols are reported to extract diosgenin [12-14]. Past research shows various methods analyzing Diosgenin such as microscopy [5], spectrophotometric [15], GLC [16], and HPTLC [17-20], UHPLC-QTOF-MS [20-21].

Materials and Methods

Raw Material: *Dioscorea* germplasm (Fourteen accessions) comprising seven distinct species was collected from wild sources of Eastern Ghats of Odisha by scientists of ICAR-NBPGR through explorations from tribal areas of Odisha situated in Eastern Ghats of India. These have been maintained at Field Gene Bank of NBPGR Regional Station, Cuttack. The tribal inhabitants use the dioscorea tubers as food and also as herbal medicines to treat different diseases (Table 1). These *Dioscorea* species studied in present studies were: *D. bulbifera* L. (2), *D. pentaphylla* L. (4), *D. hispida* Dennst. (1), *D. alata* L. (3), *D. oppositifolia* L. (2), *D. pubera* Blume (2), *D. hamiltonii* Hook. F. (1). Tubers were dried in horizontal tray dryer and pulverized to a fine powder of mesh size 40 for diosgenin extraction.

Extraction of diosgenin from *Dioscorea* tubers by Acid Hydrolysis: Diosgenin extraction and determination in *Dioscorea* tubers was carried out according to method of Trivedi[22] with slight modifications as described here. Dried tuber powder (10 g) of *Dioscorea* species was acid hydrolyzed with 50 ml of 1M ethanolic sulfuric acid at 80˚C for 8 h. The extract was filtered and extracted thrice with hexane (50 ml x 3). All the three hexane extracts were pooled and subsequently rinsed with 5% alkali three times followed by rinsing with distilled water. The extract was then passed through a column of Na_2SO_4 to eliminate any remaining water. The samples were concentrated to dryness by evaporating the solvent at 40ºC in a rotary evaporator. The samples were evaporated to dryness at room temperature and dry residues were dissolved 5 ml methanol for quantification of diosgenin by HPTLC. All the chemicals used in the studies were of analytical grade.

Table 1: Edible and medicinal uses of *Dioscorea* species by local tribes of Eastern Ghats of India

Name of Species	Local name	Parts used	Mode of preparation and uses*
D. alata L.	Kham Alu/ Khamba Alu	Tuber	Washed, sliced and cooked with other vegetables and made a mixed curry and taken with day meal. Slices added with processed rice/ gram flour, fried and made delicious cakes.
D. bulbifera L.	Pita aalu	Tuber and bulbil	Repeatedly washed, sliced, boiled and kept overnight in running water in porous bamboo container. It is further boiled to remove bitterness and allowed to cool and consumed as snacks with honey. Frequently used as food during critical times of continuous rains. Cooked and taken as curry too. Powder of dried tuber is used as contraceptive and given once a day for one week just after menses for birth control. Sometimes, the powder is added in the local wine to enhance potency.
D. hispida Dennst.	Hasar sanga Khulu sanga	Tuber	Sliced, soaked in running water and boiled successively with leaves of *Matha sag* (*Antidesma acuminata)* or tamarind to remove the acridness of tuber. The excess water is filtered out and further cooked as curry or eaten as such during food scarcity. Sometimes the tribals add the processed tubers in preparation of *Handia*, a local wine.
D. oppositifolia L.	Nai sanga, Pana alu	Tuber	Peeled and eaten raw. Cooked with other vegetables and onion and consumed as curry. Also consumed during critical periods.
D. pentaphylla L.	Bayan aalu	Tuber	Sliced, thoroughly washed and cooked with onion and spices. It is taken on the day of fasting or *Kalipuja* (a festival of Goddess *Durga*) as main meal and also consumed during food crisis. Boiled tuber is given to children to cure worm infestation in stomach.
D. puber Bl.	Kukui sanga, Kasa alu	Tuber and bulbil	Sliced and cooked to make curry or fried as cake with oil and spices. It is boiled with salt and taken also as chutney. The bulbils are eaten raw, burnt or fried as snacks during field works when hungry. Tubers are also eaten during critical periods.
D. hamiltonii Hook.f	Merem toa sanga	Tuber	Fresh tuber is slimy and tasty and eaten raw by children. Field workers in the forest also consume it when in thirst.

*Misra RC, Sahoo HK, Paani DR & Bhandari DC. Genetic resources of wild tuberous food plants traditionally used in Similipal Biosphere Reserve, Odisha, India, *Genet Resou Crop Evol,* 60 (2013) 2033-2054.

Quantitative profiling for diosgenin by HPTLC: Diosgenin analysis was done using Camag (Switzerland) HPTLC system comprising Linomat V semi-automatic spotting device, WinCATS software (Ver. 1.2.3), Scanner III and CAMAG twin-trough chamber (20 × 10 cm). Chromatography was carried out on aluminum HPTLC plates (20 × 10 cm) precoated with silica gel G60 F_{254} (E. Merck, Darmstadt, Germany). Standard diosgenin (10 mg) purchased from Sigma chemicals, USA was dissolved in methanol and diluted to obtain stock solution containing 1000 µg/ml of diosgenin. One milliliter of stock solution was further diluted to 10ml with methanol to get working standard solution of diosgenin containing 100 µg/ml of diosgenin.

The dioscorea tuber extracts (10 µL each) were applied to HPTLC plate along with 1 µl -12 µl standard diosgenin (0.1 µg/µl in methanol). The spots were applied with automatic Linomat V sample applicator, fitted with a Camag micro syringe in N_2 flow. TLC plates were developed in an ascending mode in a Camag twin-trough glass chamber using standardized pre saturation time for mobile phase was 30 minutes at room temperature (25 ± 2°C). After development, chromatographic plates were air-dried for 5 minutes. Derivatization of the spots was carried out with the derivatizing reagent anisaldehyde - sulphuric acid spray for visualization and subsequently plate was scanned. The derivatizing reagent anisaldehyde - sulphuric acid was prepared by mixing of 0.5 ml anisaldehyde reagent with 10 ml glacial acetic acid, followed by addition of 85 ml methanol and 5 ml concentrated sulphuric acid. HPTLC plates were dipped in the derivatizing reagent for 2 sec and then heated at 110°C for 10 min. Densitometric scanning was performed on Scanner III with WinCATS software in the absorbance-reflectance mode at 450 nm detection wavelength using a slit dimension 5.00 × 0.45 mm and a scanning speed of 100 nm/s. Stock solution of diosgenin standard (0.1 µg/µl) was used in different concentrations (100 ng/spot -1200 ng/spot) for preparing calibration graph of peak area versus concentration. Concentration of diosgenin in the tuber extract was calculated using this standard linear calibration equation.

Results and Discussion

HPTLC profile of *Dioscorea* species showed diosgenin spot at R_F value of 0.65 ± 0.02 **(**Fig. 2) in standardized mobile phase of Toluene: Ethyl acetate: Formic acid, (6: 5: 1, v/v) with reference to standard compound. A spectra overlay of diosgenin spot with standard compound and *Dioscorea* tuber extract at 450 nm was obtained which further confirmed presence of diosgenin in *Dioscorea* species under study. Diosgenin concentration was calculated from liner calibration curve (100-1200 ng/spot) prepared from pure diosgenin. Diosgenin content present in these *Dioscorea* species was found in the range of 0.001 to 0.003% on dry weight basis (Table 2). Though all the species showed low diosgenin content, among these *D. hispida* and *D. bulbifera* species showed slightly better diosgenin, while negligible amount were observed in other species like *D. pentaphylla* and *D. oppositifolia*. Asha and Nair (2005) reported the maximum diosgenin yield in *D. pubera* (1220 µg/g dry weight) followed by *D. spicata*, *D. hispida* and *D. hamiltonii* from Western Ghats. The low diosgenin content presently detected in above *Dioscorea* species may be due to the interaction of environmental factors or may be due to genetic makeup of the parent clones. Diosgenin content was reported from 0.048-0.133% in *D. alata* from Western Ghats by Shah and Lele (2012). The results of the current work suggest HPTLC fingerprint analysis can be used in discriminating between the *Dioscorea* species studies as the taxonomy of *Dioscorea* is confusing and identification of the species is generally problematic.

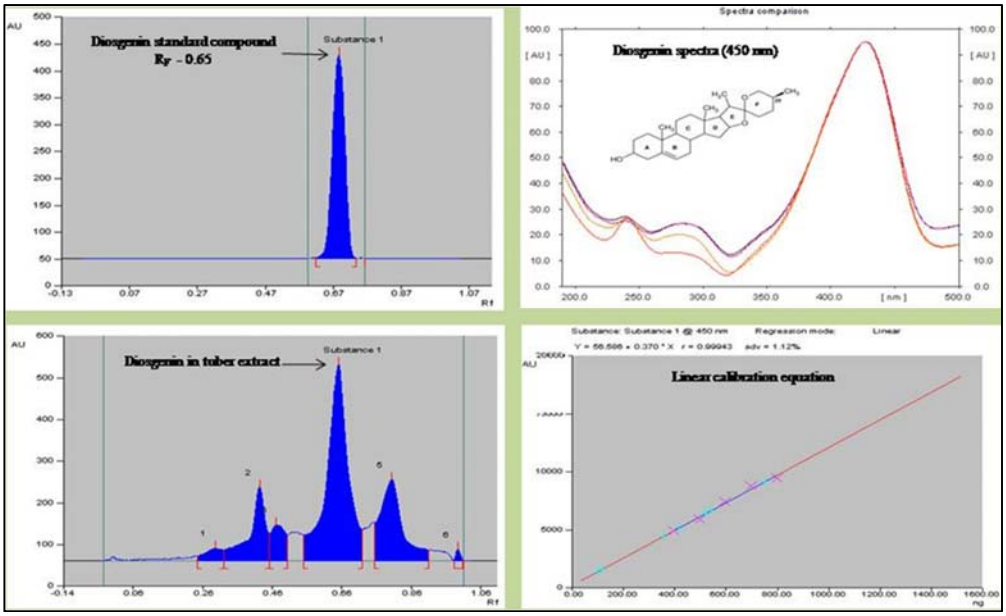

Fig 2: HPTLC separation and quantification of diosgenin in tuber extract of *Dioscorea* species

Table 2: Quantitative analysis of diosgenin content (%) in *Dioscorea* species tubers collected from wild areas in Eastern Ghats of Odisha

Collector No.	Botanical name	Vernacular name	Diosgenin (%)	Type of material	Source	Collection site			
						Village	Mandal	District	State
RCM/AP/16	*Dioscorea bulbifera*	Pita alu	0.0021	Tuber	Disturbed wild	Kasipani	Bisoi	Mayurbhanj	Odisha
RCM/AP/63	*Dioscorea bulbifera*	Pita alu	0.0023	Tuber	Natural wild	Baghalata	Karanjia	Mayurbhanj	Odisha
RCM/AP/21	*Dioscorea pentaphylla*	Bayan alu	0.0005	Tuber	Natural wild	Kasipani M. I. proj.	Bisoi	Mayurbhanj	Odisha
RCM/AP/17	*Dioscorea pentaphylla*	Bayan alu	0.0012	Tuber	Natural wild	Kasipani nala	Bisoi	Mayurbhanj	Odisha
RCM/AP/60	*Dioscorea pentaphylla*	Bayan alu	0.0004	Bulbil	Farmer's field	Balibod	Karanjia	Mayurbhanj	Odisha
RCM/AP/81	*Dioscorea pentaphylla*	Bayan alu	0.0014	Tuber	Natural wild	Chakratirtha	Anandpur	Keonjhar	Odisha
RCM/AP/18	*Dioscorea alata*	Kham alu	0.0012	Tuber	Fallow	Kasipani	Bisoi	Mayurbhanj	Odisha
RCM/AP/50	*Dioscorea alata*	Kham alu	0.0009	Tuber	Fallow	Chidiki	Bangiriposi	Mayurbhanj	Odisha
RCM/AP/61	*Dioscorea alata*	Kham alu	0.0015	Tuber	Farmer's field	Balibod	Karanjia	Mayurbhanj	Odisha
RCM/AP/19	*Dioscorea puber*	Kasa alu	0.0003	Tuber	Natural wild	Kasipani nala	Bisoi	Mayurbhanj	Odisha
RCM/AP/37	*Dioscorea puber*	Kasa alu	0.0015	Tuber	Natural wild	Ghatkumari	Bisoi	Mayurbhanj	Odisha
RCM/AP/20	*Dioscorea hamiltonii*	Chuna alu	0.0007	Tuber	Natural wild	Durdura kacha	Bisoi	Mayurbhanj	Odisha
RCM/AP/23	*Dioscorea oppositifolia*	Pana alu	0.0008	Tuber	Natural wild	Kasipani M. I. proj.	Bisoi	Mayurbhanj	Odisha
RCM/AP/73	*Dioscorea oppositifolia*	Pana alu	0.0012	Tuber	Natural wild	Kendudia	Ghatgaon	Keonjhar	Odisha
RCM/AP/56	*Dioscorea hispida*	Bayan alu	0.0027	Tuber	Natural wild	Bangiriposi ghati	Bangiriposi	Mayurbhanj	Odisha

Conclusion

The edible tubers from different species of *Dioscorea* are a major source of food and nutrition for millions of people. Some of the species are medicinally important. The proposed quantitative method for analysis of Diosgenin by HPTLC is simple, faster and cost-effective. Among the tuberous wild edible medicines, *Dioscorea species* are quit common. They are a prime staple food substitute for the majority of rural and local people of the plant parts are quite useful in treatment of different types of diseases and disorders due the presence of a numbers of bioactive compounds. The most important identified compound from *Dioscorea species* is diosgenin, it is presently used in the synthesis of steroidal drugs; however other potential uses of this compounds and related compounds as estrogenic, anti-inflammatory and anticancer potential need to be studied extensively. Studies should also be carried out to utilize the bioactive compounds present in these tubers for formulation of new drugs to fight against pathogenic multidrug resistant microorganisms and antimicrobial resistance. The ethno medicinal potential of various plant species under this genus need to be validated and detailed investigations on the composition and pharmacological significance of the medicinal plants under this genus along with the standardization of the formulations used should be undertaken extensively.

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